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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF *HYPTIS SUAVEOLENS* EXTRACT AND ITS EFFECTS ON THE GROWTH OF DISEASE-CAUSING PARASITES

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Abstract

Plants extracts contain many essential natural synthesized chemical compounds with considerable potentials for medicinal exploitation and application. There has been a growing concern about the adverse effects of mosquito repellants and the need to search for natural and environment-friendly Mosquito repellants. Synthetic insecticides and their associated toxicity issues and the growing incidence of insect resistance have inspired novel insecticides. The present study analyzed the phytochemical extract of *H. Suaveolens* its effect on mosquito pupa. A total number of pupa (320) was poured into a 200ml glass beaker; twenty pupae each per three hours was introduced into a glass beaker containing different extract concentrations (ppm) of *Hyptis suaveolens*. Twenty pupae were introduced in contrast to a glass beaker containing 100ml of distilled water treated with 1ml of acetone used as treated control. Twenty pupae were introduced into 100ml of distilled water and used as untreated control. The result reveals that *Hyptis suaveolens* possesses inhibitory activity against Anopheles gambiae

Introduction: -

Plant extracts are a rich source of naturally synthesized chemical compounds (Muthukrishnan et al., 2014). The concept of developing drugs from plants in the indigenous medical system is much older (Mishra et al., 2011).The traditional population uses *Hyptis suaveolens* several parts of the world to treat illnesses (Barbosa et al., 2013; Jesus et al., 2013; Vera-Arzave et al., 2012). It has been reported to relieve respiratory and gastrointestinal infections, indigestion, cold, pain, fever, cramps, skin diseases, gastric ulcers, and inflammatory disorders (Machado et al., 2021). The extracts from *H. suaveolens* show considerable potential for medicinal exploitation and application (Li et al., 2020). For instance, the plant has been found helpful in antimicrobial and antioxidant activity (Das et al., 2017; Gavani&Paarakh, 2008; Nantitanon et al., 2007), parasitical cutaneous diseases (Pachkore & Dhale, 2011), neuroprotective activities (Ghaffari et al., 2014), antiviral activity (Kothandan & Swaminathan, 2014), antimycobacterial and cytotoxic activities (Aremu et al., 2020; Mandal et al., 2007; Prawatsri et al., 2013; Satish et al., 2010), antiplasmodial activity (Chukwujekwu et al., 2005), antifungal (Moreira et al., 2010), wound healing activity (Bayala et al., 2020; Shenoy et al., 2009), antidiarrhoeal activity (Shaikat et al., 2012), antinociceptive (Santos et al., 2007). However, biological attributes of the plant have been well documented owing to its good medicinal value due to the presence of essential oils, alkaloids, flavonoids, phenols, saponins, terpenes, and sterols. In traditional medicine, the plant leaves are applied as insectifuge because of their intense aroma, especially against mosquitoes.

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Mosquitoes are a significant global public health concern, with a concomitant increase in people at risk of infection (Guégan et al., 2018). It has become the oldest human enemy and represents a significant threat to human health because of its ability to vector pathogens that cause diseases like Dengue fever, Dengue hemorrhagic fever, Malaria, Japanese encephalitis, and Filariasis that afflict millions of people worldwide. (Lawler and Lanzaro 2005). Mosquito-borne diseases are associated with significant global health burdens (Lee et al., 2019).

There has been a growing concern about the adverse effects of Mosquito repellants. There has been an increasing need to search for natural and environment-friendly mosquito repellants (Singh et al., 2011). Synthetic insecticides and their associated toxicity issues and the growing incidence of insect resistance have inspired novel insecticides. Plant extracts may be alternatives sources that constitute a rich source of bioactive compounds that are biodegradable and environmentally friendly. Research has shown that *H. suaveolens* an invasive weed with insecticidal properties (Benelli et al., 2012; Devi Priya, 2016; Sharma et al., 2019). Thus, the current study intends to explore the inhibitory role of *H. suaveolens* aqueous extract on the pupa of mosquitoes.

Materials and Method: -

Collection of Anopheles mosquito pupa

The anopheles' mosquito pupa was collected from stagnant waters at various locations in Kogi state using dipper and pipette as described. The collected pupa was free from tadpoles which may cause its mortality. The pupa was reared in a clean white plastic bowl covered with a fine and clean mosquito net.

Plant source

The plant *Hyptis suaveolens* leaves were collected and confirmed by a Botanist. The leaves were washed with distilled water and sliced into small pieces, and dried.

Preparation of Crude Extract

Soxhlet Extraction

The crude extraction of *Hyptis suaveolens* was done using the Soxhlet extraction technique as describe by Zygler et al. (2012). The coarse powder (200g) of *Hyptis suaveolens* leaves was gained by pounding the air-dried leaves with a mortar and pestle. The coarse powder was transferred into the Soxhlet extractor column and measured into the flat bottom flask and the Soxhlet. A reflux condenser was inserted into the Soxhlet, and rubber hoses connected from the condenser to the circular water. The setup was placed in a heating mantle, and the mantle was connected to the main connection.

Preparation of Test Concentration

Plant crude extract was prepared through a single dilution method by mixing it with distilled water, each in a sterile glass beaker. Treated control was prepared and kept safe before pupa inhibitory bioassay.

Pupa Inhibitory Bioassay

Pupa bioassay will be prepared according to a standard procedure provided by the world health organization, guideline for laboratory and field testing of mosquito pupacide (WHO, 2005). The pupa was transferred through strainers of droppers to labeled sterile glass beakers of concentrations. The pupa inhibitory activities of each extract concentration were evaluated by counting the number of dead pupae for the period. Pupa was confirmed dead when no movement is observed and no response to a stimulus when touched with a pasture pipette. The dead pupa was carefully removed from the setup and placed in a clean filter paper, counted, and recorded at some intervals.

Result: -

Tables containing the number of dead and live pupa against each concentration were made. Degree activities were evaluated by plotting the number of dead pupae against concentration. The percentage of dead and alive for every interval will be determined.

Table 1: - Table showing the effect of different concentrations of the crude extracts of *H. suaveolens* on Anopheles gambiae pupa at a different time interval.

Extract concentration	Number of pupa exposed	Number of pupa died in 3hours	Number of dead pupa in 9hours	Number of dead pupa in 12hours (PPM)
Treated Pupa	20	0	0	0

Untreated pupa	20	0	0	0
50ppm	20	2	5	11
100ppm	20	3	6	15
150ppm	20	5	8	17
200ppm	20	9	11	19

Table 2: - Table showing the effect of different concentrations of extracts (ppm) and percentage % death and alive in 3hours.

Extract concentration (ppm)	Number of pupa exposed	Number of pupa died in 3hours	(%) dead	(%) alive
Treated Pupa	20	0	0	0
Untreated pupa	20	0	0	0
50ppm	20	2	10	90
100ppm	20	5	15	85
150ppm	20	5	25	75
200ppm	20	9	45	55

Percentage (%) dead = $\frac{\text{n dead after treatment}}{\text{n in control}} \times \frac{100}{1}$

(Percentage (%) alive = 100 – percentage (%) dead)

Table 3: - Table showing the different concentrations of extracts (ppm) and percentage % death and alive in 6hours.

Extract concentration (ppm)	Number of pupa exposed	Number of pupa died in 6hours	(%) dead	(%) alive
Treated control	20	0	0	0
Untreated control	20	0	0	0
50ppm	20	5	25	75
100ppm	20	6	30	70
150ppm	20	8	40	60
200ppm	20	11	55	45

Table 4: - Table showing the different concentrations of extracts (ppm) and percentage % death and alive in 9hours.

Extract concentration (ppm)	Number of pupa exposed	Number of pupa died in 9hours	(%) dead	(%) alive
Treated control	20	0	0	0
Untreated control	20	0	0	0
50ppm	20	9	45	55
100ppm	20	10	50	50
150ppm	20	13	65	35
200ppm	20	15	75	25

Table 5: - Table showing the different concentrations of extracts (ppm) and percentage % death and alive in 12hours.

Extract concentration (ppm)	Number of pupa exposed	Number of pupa died in 12hours	(%) dead	(%) alive
Treated control	20	0	0	0
Untreated control	20	0	0	0
50ppm	20	11	55	45
100ppm	20	15	75	25
150ppm	20	17	85	15
200ppm	20	19	95	5

Discussion: -

Natural pesticides, especially those derived from plants, are more prosing and effective in mosquito (pupa) control(Amer & Mehlhorn, 2006). *Hyptis suaveolens* were found to have some pupa inhibitory activity against

mosquito pupa at different concentration rates in part per million (ppm), as shown in table 1. The result showed the net change in the death rate of pupa with a subsequent increase in its concentration compared to control. A total number of pupa (320) was poured into a 200ml glass beaker; twenty pupae each per three hours was introduced into a glass beaker containing different extract concentrations (ppm) of *Hyptis suaveolens*. Twenty pupae were introduced in contrast to a glass beaker containing 100ml of distilled water treated with 1ml of acetone used as treated control. Twenty pupae were introduced into 100ml of distilled water and used as untreated control.

Table1 shows the effect of different concentrations of crude extracts of *Hyptis suaveolens* on *Anopheles Gambiae* larvae at different time intervals. After 12hours, no motility was recorded for treated and untreated control. In three (3) hours, ±2 pupa was recorded dead against a concentration of 50ppm. After three hours, twenty (20) pupa was introduced into another glass beaker containing 50pm of crude extract and allowed to stand. In six (6) hours, ±5 pupa was recorded dead. The same procedure was repeated for the 9^{th} and 12^{th} hours, respectively. In the 9^{th} hour, ±9 pupa was recorded dead, and in the 12^{th} hour, ±11 pupa was recorded dead. The same procedure was repeated for concentrations 100ppm, 150ppm, and 200ppm. At concentration 100ppm, in 3hours, 6hours, 9hours and 12hours, ±3, ±6, ±10, and ±15 pupa was recorded dead, respectively. At concentration 150ppm, in 3hours, 6hours, 9hours, and 12hours, ±5, ±8, ±13 and±17 pupa was recorded dead. At concentration 200ppm, in 3hours, 6hours, 9hours and 12hours, ±9, ±11, ±15, and ±19 pupa was recorded dead. Percentage of the dead for pupa that died in 3hours, 6hours, 9hours, and 12hours were calculated by dividing the number of pupae dead after treatment with several controlled (20) and multiplying with 100%, and percentage alive for the remaining pupa was calculated by subtracting percentage dead from 100%. This procedure was used in the following tables. The inhibitory activity was found to be higher against the pupa at the highest concentration rate of 200ppm.

Conclusion: -

Plant extracts in insect/mosquito control are an effective pest control method and help minimize the aggressive growth of mosquitoes. The result reveals that *Hyptis suaveolens* possesses inhibitory activity against Anopheles gambiae. Therefore, the result contributed to the literature by laying the ground for further analysis of the bioactive constituents of *Hyptis suaveolens* extract and its systemic effects on target mosquitoes. This may enable the application of the extract as a pupa inhibitor in a considerable area of aquatic habitats or breeding sites in and around human dwellings for effective control of vector mosquitoes.

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